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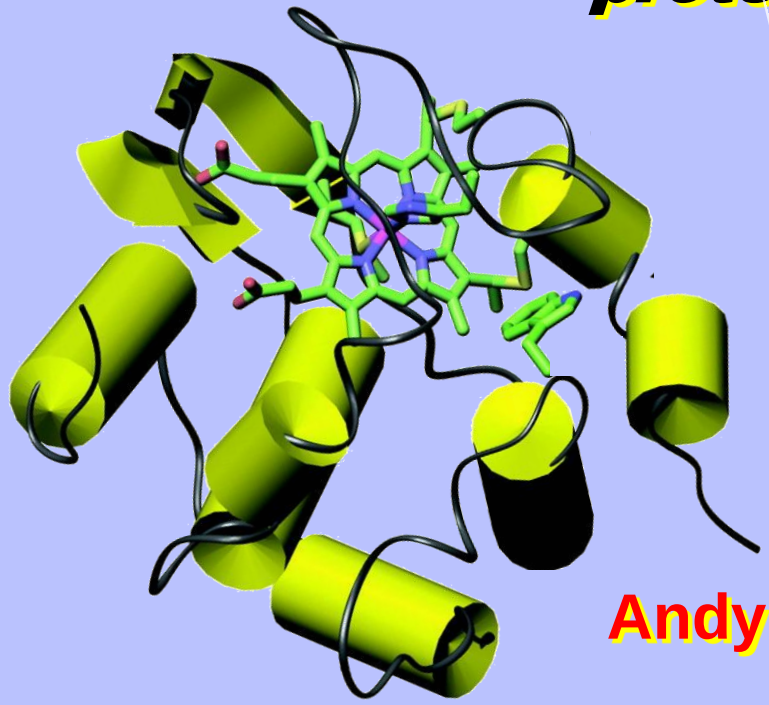
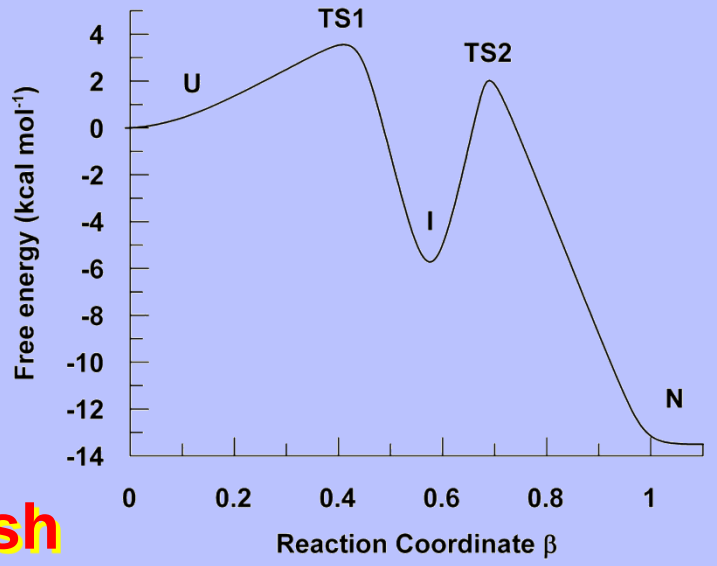
Suggestions for experimental work concerning protein folding





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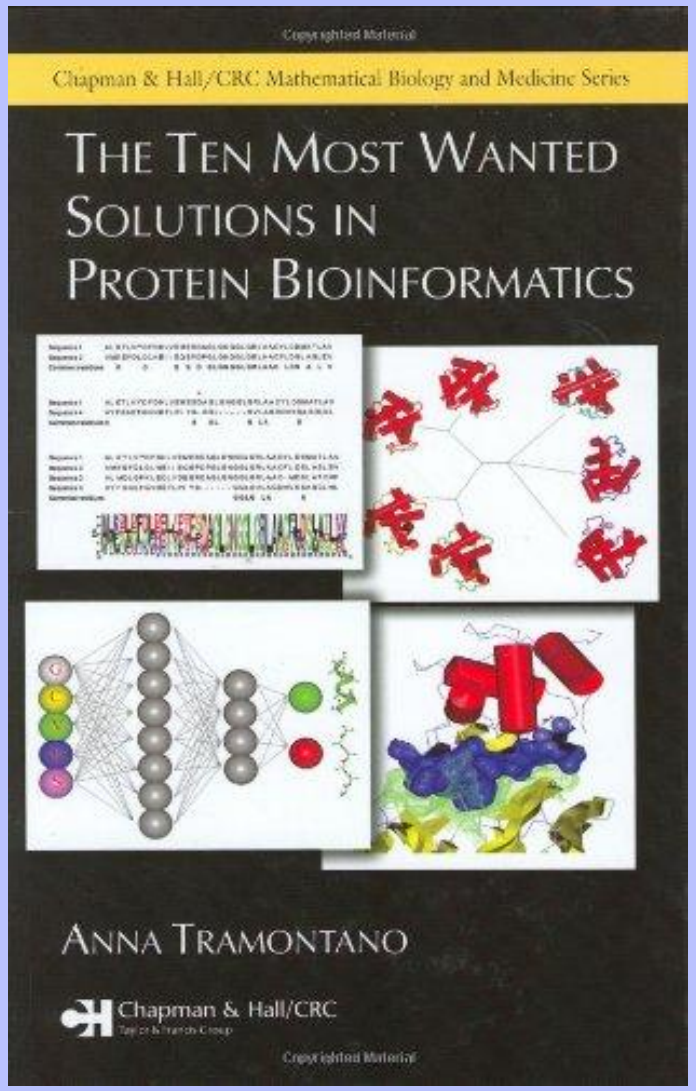
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INFORMATION AND ENTROPY – TOP-DOWN OR BOTTOM-UP DEVELOPMENT IN LIVING SYSTEMS?

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Exploring the Cytochrome *c* Folding Mechanism

CYTOCHROME c_{552} FROM *THERMUS THERMOPHILUS* FOLDS THROUGH AN ON-PATHWAY INTERMEDIATE*

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Maynard Smith and Szathmary [1] discuss the folding of proteins once they have formed initially as a string of amino acids coded for by the genes in the DNA. In their discussion of pattern formation they draw an analogy with a picture made by an ink-jet printer. They state "..a protein molecule is made in a way analogous to such an image. Thus, there is a one-to-one correspondence between amino acids in the protein and base triplets in the gene that coded for it. Change one base and you will change one amino acid. This is not the whole story: the gene specifies the sequence of bases in the protein, but the string must then fold up to produce the three-dimensional form. In most cases, the string will fold up on its own: folding is a self-organised dynamic process depending on the laws of physics, which do not need to be programmed"

Is this always the case??

[1] Maynard Smith, J. & Szathmary, E., The Origins of Life, Oxford University Press: Oxford, New York, 1999.

Questions to consider :

a) What are the free energy pathways involved in protein folding?

b) Are they necessarily always entirely predictable?

c) What is the role of compact on-pathway intermediates in folding such as with Cytochrome (cyt) c_{552} ? Could the Tryptophan (Trp) intermediates possibly be chaperones?

d) Can the free energies at each stage be directly measured?

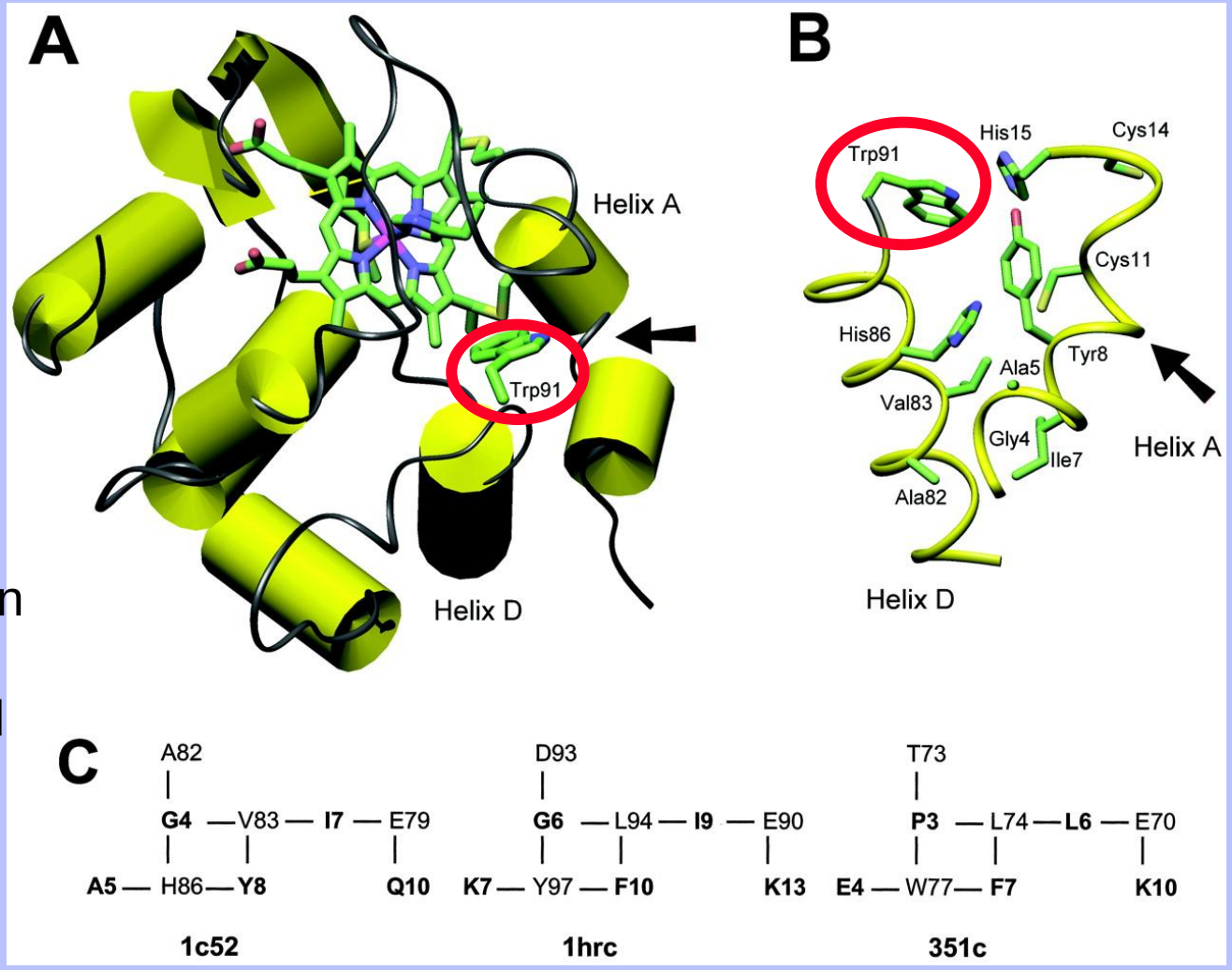
e) Are there experiments which can be done to explore the free energy phase space of possible energy bonding?

"The mechanism by which an unfolded polypeptide chain finds its unique native state is one of the most intriguing problems in biology Extensive kinetic studies led to the hypothesis that protein folding proceeds along a defined reaction pathway whereby the polypeptide is driven through one or more partially structured intermediates."

Travaglini-Allocatelli, C., Gianni, S., Morea, V., Tramontano, A., Soulimane, T. and Brunori, M., "Exploring the Cytochrome c Folding Mechanism", Journal of Biological Chemistry, Vol. 278, No. 42, Issue of October 17, pp. 41136–41140, 2003

Three-dimensional structure of cyt c552 from T. *thermophilus*

Fluorescence emission of the single Trp at position 91 (quenched by the hemeprotein in the native state)



Travaglini-Allocatelli C et al. J. Biol. Chem. 2003; 278:41136-41140

Refolding and unfolding initiated by a symmetric mixing of the denatured or the native protein with the appropriate buffer. Unfolded cyt *c*552 obtained by incubation in 5 M GdnHCl at pH 2.1 (5 moles of Guanidine Hydrochloride)

Equilibrium experiments:- Assuming a standard two state model, the GdnHCl-induced denaturation transitions were fitted by Travaglini-Allocatelli et. al. to the equation

$$\Delta G_d = \Delta G_W - m_{UN} .D$$

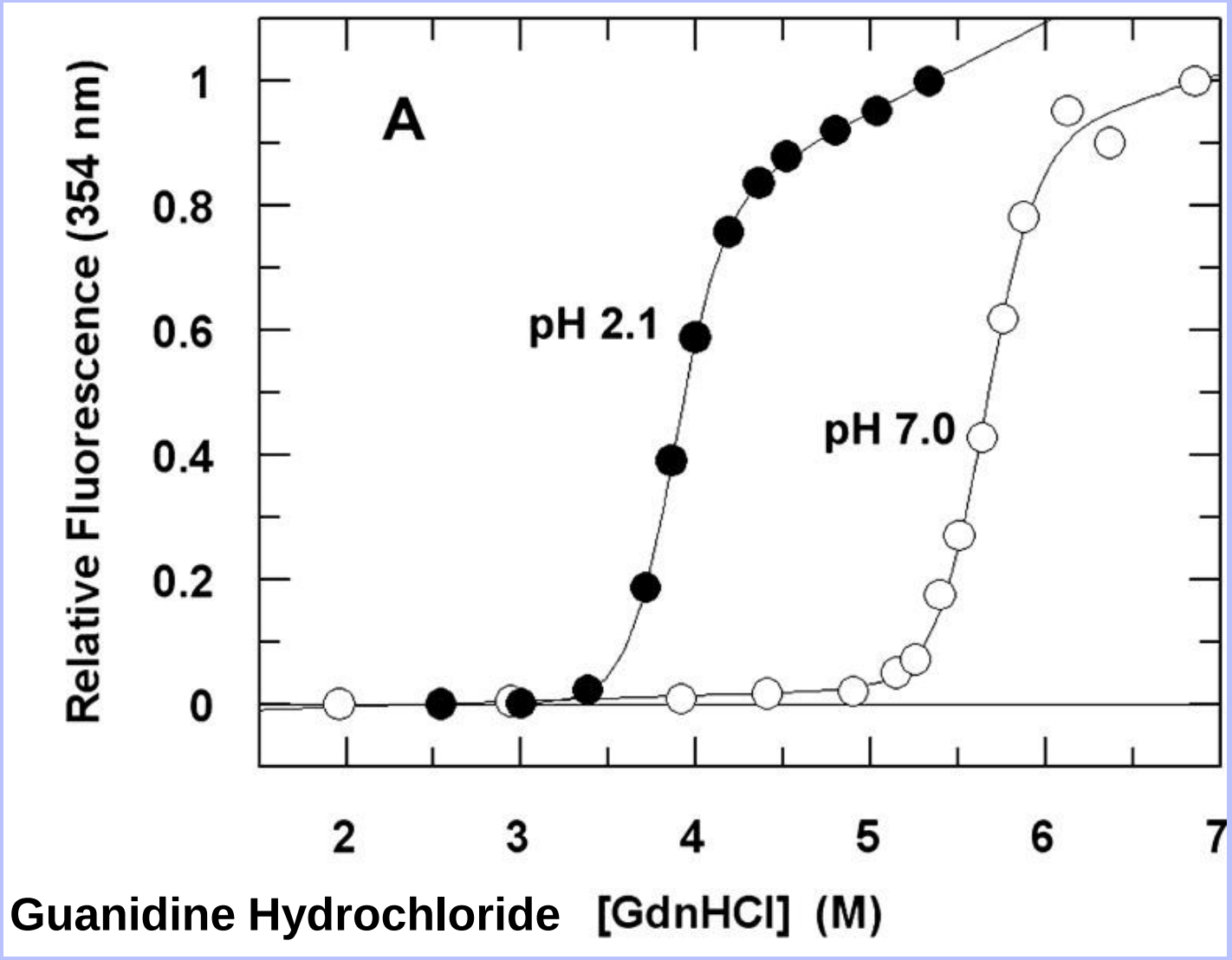
where $\Delta G_d = \Delta G_W$ are the free energy of folding in water and at a concentration *D* of denaturant.

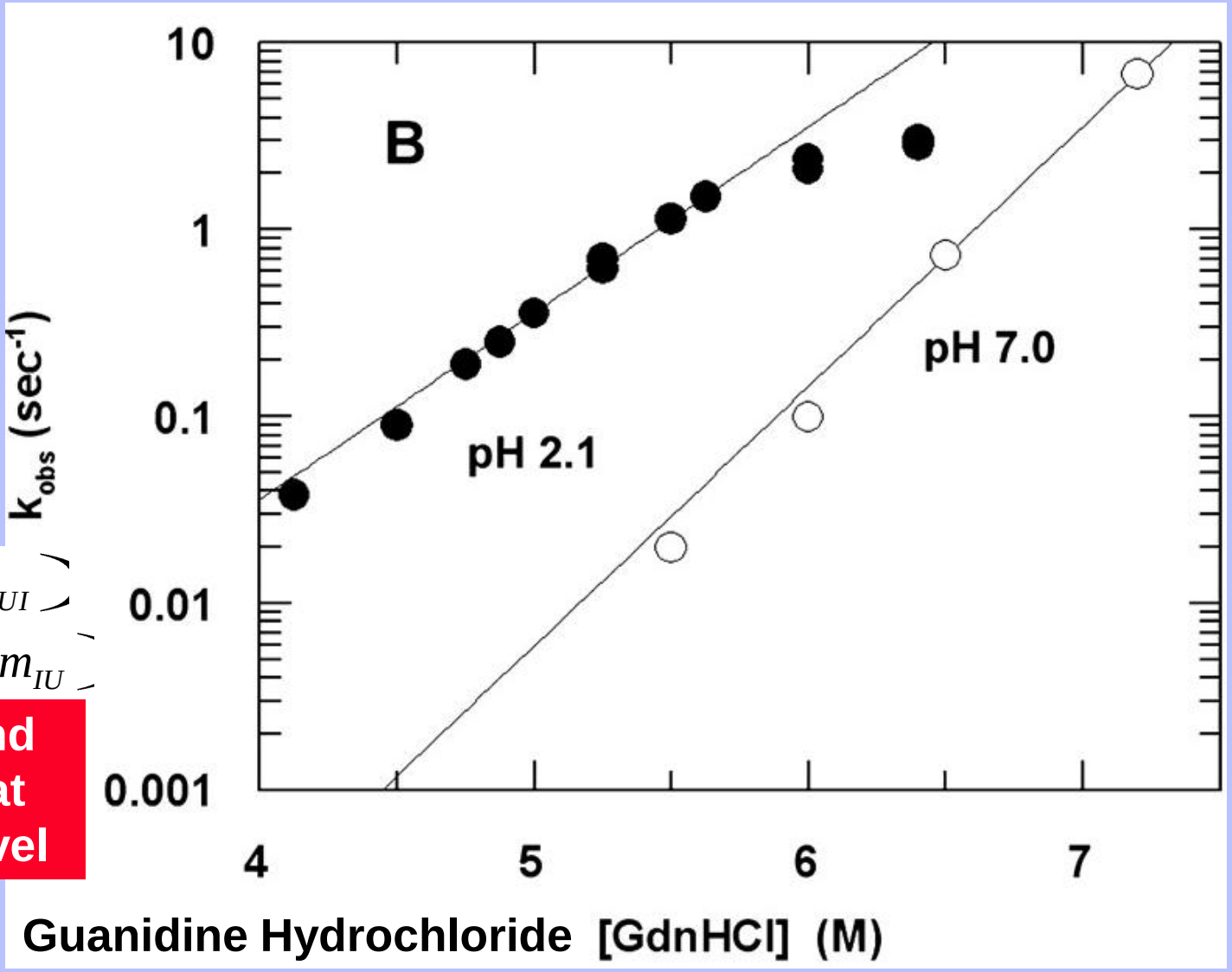
Two state model :

$$U \underset{K_{UI}}{\overset{K_{UI}}{\Leftrightarrow}} I$$

$$k_{UI} = k_{UI}^0 \exp\left(Dm_{UI}\right)$$

$$k_{IU} = k_{IU}^0 \exp\left(-Dm_{IU}\right)$$


A
pH 2.1
pH 7.0
Relative Fluorescence (354 nm)
0
0.2
0.4
0.6
0.8
1
2
3
4
5
6
7
Guanidine Hydrochloride [GdnHCl] (M)

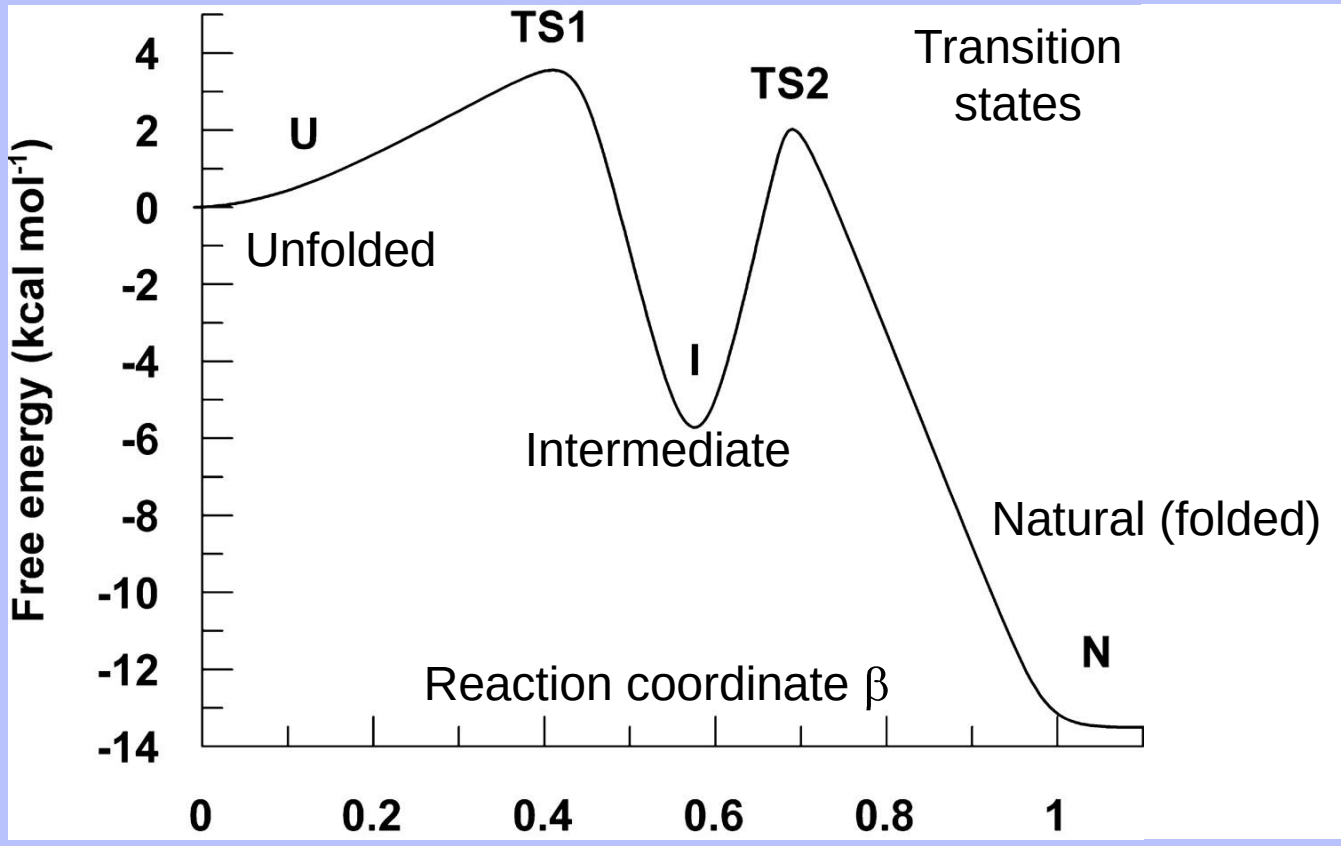


$$k_{UI} = k_{UI}^{0} \exp(m_{UI})$$

$$k_{IU} = k_{IU}^{0} \exp(m_{IU})$$

Curve fits to find reaction rates at any GdnHCl level

Free Energy diagram



The reaction coordinate β represents the fractional burial of solvent-accessible surface area calculated as follows:

$\beta_{TS1} = m_{UI}/(m_{UI} - m_{IU} + m_{IN} - m_{NI})$; $\beta_I = (m_{UI} - m_{IU})/(m_{UI} - m_{IU} + m_{IN} - m_{NI})$;

$\beta_{TS2} = (m_{UI} - m_{IU} + m_{IN})/(m_{UI} - m_{IU} + m_{IN} - m_{NI})$.

Possible diagnostic experiments with x rays :

1) Could x-rays be passed through the proteins and intermediates to ascertain the energy bonds as the protein is folded? [This would be a more direct measurement rather than by inference with flouresence emission intermediate].

2) A deeper question - Is the folding really predictable chemically, or could there be a response to a subtle coding – particularly to overcome the Free Energy compact peaks? Could the Tryptophan (Trp) intermediates possibly be chaperones programmed for the main protein (Cytochrome)?

3) Could such x-ray experiments be done to explore the free energy phase space?

Thank you

Any questions and discussion?